NITROGENOUS EXCRETION IN AQUATIC AND TERRESTRIAL CRUSTACEANS

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For water-breathing animals the most economical nitrogenous excretory product in energetic terms is ammonia (ium) and this is generally excreted across the gills. In terrestrial situations most animals produce less toxic materials, notably urea and purines, which are eliminated by the excretory organs. Crustaceans are predominantly an aquatic group and marine and freshwater species conform to the above pattern in excreting ammonia (ium). Most amphibious species generally have frequent recourse to water and show little change in the basic aquatic pattern of excretion. The small terrestrial forms, notably Amphipoda and Isopoda and the crab *Geograpsus grayi*, retain ammonia excretion but eliminate it as a gas. The gecarcinid land crabs excrete NH_4^+ in the urine whilst *Birgus latro* excretes uric acid in the faeces. Many terrestrial and some aquatic species store purines and may have purine synthetic ability. The role of stored purine is unclear but it may act as a N reserve, an ion store or as a non-toxic buffer when normal ammonia excretion is inhibited. Urea is not an important excretory product and crustaceans appear to lack a complete urea cycle. Excretory patterns are discussed in relation to the evolutionary history of the group. □

Nitrogenous excretion, Crustacea, ammonia, terrestrial Crustacea, uric acid

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The form in which animals excrete waste nitrogen and the route, or mechanisms, by which it is eliminated are quite labile, but there are several underlying factors which strongly affect the mechanism employed. Firstly, metabolism of protein yields ammonia[1], typically from transamination and deamination reactions, and the metabolically cheapest form of excretion is ammonia. Secondly, ammonia is toxic to animals, especially higher vertebrates (Campbell, 1973), although less so to crustaceans which may maintain ammonia concentrations appreciably higher than in vertebrates (Table 1). Thirdly, NH_3 is highly diffusible and cannot be contained or concentrated by cells, but in the ionic form it can be concentrated and transported by the usual ion transport mechanisms. In body fluids most ammonia is protonated (NH_4^+) and within the usual range of pH only around 1% will be present as NH_3. Elevated $[NH_4^+]$ however, leads to elevation of $[NH_3]$ with resultant toxic effects, so that ammonia concentrations must be kept within a tolerable range. Rapid excretion into large volumes of water avoids any build up in concentration. For these reasons, ammonia excretion is considered to be the preserve of water-breathers which can economically excrete their waste in the form in which it is produced.

Other forms of waste nitrogen are also eliminated by animals, chiefly purines and urea, but these have several selective disadvantages. They are more complex molecules and their synthesis requires energy and also elaborate enzyme systems (Hartenstein, 1968). This extra metabolic cost is only outweighed under conditions considered inadequate for safe excretion of ammonia, i.e. on land, especially in mesic and xeric habitats where water availability is limited.

The generalised pattern seen in animals then is as follows: water-breathers are ammonotelic excreting NH_3/NH_4^+ across suitable surfaces, usually the gills; terrestrial forms (particularly arthropods) excrete purines or, less commonly, urea, and any NH_3/NH_4^+ excretion is small and is often primarily a function of acid-base regulation.

The Crustacea are predominantly an aquatic group with the major radiation in the sea but with a substantial number of species in freshwater and only a few on land. Most species have spent their whole evolutionary history in water and ammonotelism is expected to be the standard pattern of excretion. The terrestrial forms would be expected to excrete one of the more complex, less toxic, compounds. The extent to which these predictions hold is examined below. Whilst water availability in the habitat is normally an

[1] In this review NH_3 refers to molecular ammonia, NH_4^+ to ammonium ions, and ammonia to the sum of both (=total ammonia).

TABLE 1. The concentration of ammonia in the haemolymph of a range of crustaceans.

Species	Ammonia $mmol.L^{-1}$	pH	Source
Callinectes sapidus	0.39	7.98	Cameron and Batterton (1978)
Callinectes sapidus	0.011 NH_3 0.82 NH_4^+		Kormanik and Cameron (1981b)
Carcinus maenas	0.9		Binns (1969)
Uca pugilator	20.0		Green *et al.* (1959)
Cardisoma carnifex	1.6	7.49	Wood *et al.* (1986)
Geograpsus grayi	2–3	7.59	Greenaway and Nakamura (in press)
Gecarcoidea natalis	2–4	7.55	Greenaway and Nakamura (in press)
Cherax destructor	0.1		Fellows and Hird (1979)
Notostomus gibbosus	217	7.52	Sanders and Childress (1988)
Porcellio scaber	1.5		Wieser and Schweizer (1972)

over-riding determinant of the excretory mechanism employed, there are certain other factors which may be important in determining excretory rates and patterns in Crustacea. These include the moult (at which time there is considerable catabolic and anabolic activity involving proteins), the nitrogen content of the diet and metabolic rate (including effects of temperature and season). Additionally, where ammonium is excreted in exchange for cations in the water the salinity of the latter may be important as osmoregulatory requirements may conflict with those for nitrogenous excretion. This may affect migratory species such as *Eriocheir sinensis* and prawns as well as species subject to fluctuating salinities (Regnault, 1987). The taxonomic inheritance in terms of available metabolic pathways and the length of time a species has spent in a particular habitat can also exert a major influence. This review is directed towards the effects of adaptation to terrestrial habitats on excretory mechanisms.

EXCRETION IN AQUATIC HABITATS

The formation, detoxification and elimination of ammonia by aquatic crustaceans has been the subject of several recent reviews (Kormanik and Cameron, 1981a; Evans and Cameron, 1986; Regnault, 1987) and whilst the subject is far from being totally understood available data are well documented. In consequence, nitrogenous excretion in aquatic crustaceans will be reviewed to provide a basis for the logical treatment of terrestrial groups.

AMMONIA FORMATION

Ammonia for excretion originates overwhelmingly from the catabolism of amino acids but minor amounts will result from the degradation of purines, pyrimidines, and urea of which the latter may originate from a high intake of arginine (Fig. 1).

Before the carbon skeleton of an amino acid can be utilised in the citric acid cycle its amino group must first be removed. Most commonly this is achieved by transamination, the transfer of the nitrogen containing α-amino group to an α-keto acid. The general reaction is as follows

$$\text{L-}\alpha\text{-amino} + \alpha\text{-ketogluterate} \underset{\text{transaminase}}{\overset{\text{amino acid}}{<=\!=\!=>}} \alpha\text{-keto acid} + \text{L-glutamate}$$

These reactions do not release ammonia but pass it on to an α-keto acid to form L-glutamate. Certain amino acids possess other N groups and these may be removed by deamination followed by transamination of the amino nitrogen e.g. the amide groups of asparagine and glutamine.

$$\text{Asparagine} + H_2O \xrightarrow{\text{asparaginase}} \text{Aspartate} + NH_4^+$$

$$\text{Glutamine} + H_2O \xrightarrow{\text{glutaminase}} \text{L-glutamate} + NH_4^+$$

These pathways are believed to be utilised in some crustaceans (Krishnamoorthy and Srihari, 1973; King *et al.*, 1985). Despite some controversy it is clear that glutamate itself can undergo oxidative deamination in crustaceans (Chaplin *et*

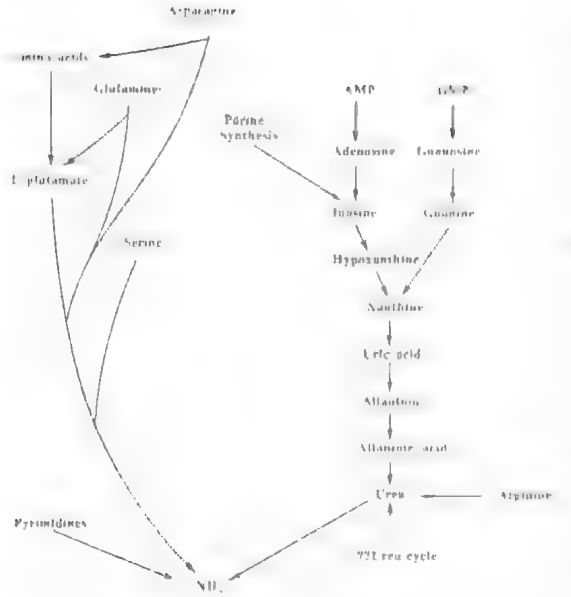

FIG. 1. Metabolic pathways producing ammonia in crustaceans.

al., 1965; Bidigare and King, 1981; Batrel and Regnault, 1965; Regnault and Batrel, 1987).

$$\text{L-glutamate} + H_2O \xrightleftharpoons{\text{GDH}} \alpha\text{-ketoglutarate} + NH_4^+$$

Indeed as glutamate is the only amino acid from which the α-amino group can rapidly be removed to release ammonia and the enzyme, glutamate dehydrogenase is very important in ammoniogenesis. In many animals, the direction of the reaction is determined by the co-factor so that oxidative deamination (NH_3 release) is stimulated by NAD^+ whilst the reducing reaction (NH_3 uptake) requires NADPH (Lehninger, 1982).

$$H_2O + \text{L-glutamate} + NAD^+ \xrightleftharpoons{\text{GDH}} NH_4^+ + NADH + H + \alpha\text{-ketoglutarate}$$

$$\alpha\text{-ketoglutarate} + NADPH + H^+ + NH_4^+ \xrightarrow{\text{GDH}} NADP^+ + H_2O + \text{L-glutamate}$$

In crustaceans the relative concentration of NAD^+ and NADH, and substrate concentrations may determine whether formation or deamination of glutamate occurs (Batrel and Regnault, 1985; Regnault and Batrel, 1987). In most cases N is transferred to glutamate and its oxidative deamination will release ammonia into the cell.

Ammonia may also be generated by catabolism of other nitrogenous compounds. Thus purine and pyrimidine nucleotides may be broken down to yield ammonia (Fig. 1), although the amounts involved are likely to be very small given the presence of scavenging pathways. Dietary intake of these components may necessitate some nitrogenous excretion, depending on the animal's requirements. In actively working skeletal muscle, the deamination of AMP results in ammonia production. Whilst AMP-deaminase has been identified in certain crustaceans its function is yet to be established (Regnault, 1987) and the importance of the pathway in overall ammonia production in crustaceans is unknown. All these pathways lead to uric acid which may then be excreted as urate, stored or degraded to urea or ammonia.

Ammonia may also be produced from urea via urease, but as a complete synthetic pathway for urea is lacking in the crustaceans examined (Hartenstein, 1970; Claybrook, 1983; Regnault, 1987) available urea is likely to be restricted to that produced from dietary arginine via arginase which is present in the midgut gland and gills of many crustaceans.

$$\text{Arginine} + H_2O \xrightarrow{\text{arginase}} \text{urea} + \text{ornithine}$$

Arginine is an essential amino acid in crustaceans that have been investigated (Zandee, 1966; Claybrook, 1983) so that urea from this source represents a dietary excess rather than a controllable form of N excretion. Urea will also be formed as an intermediary compound in uricolysis. Thus the possible generation of ammonia from urea is small.

AMMONIA DETOXIFICATION

In aquatic Crustacea, the major site of excretion is the gills (Kormanik and Cameron, 1981a; Evans and Cameron, 1986) so the ammonia generated in metabolising cells must be transported there for elimination. As the concentration of ammonia in haemolymph is high (Table 1), considerable amounts may be carried in this form. However, if production was high or excretion was discontinuous, a non-toxic molecule would be necessary for transport or temporary storage of nitrogenous waste. The obvious candidates are glutamate, the end product of transamination reactions, and glutamine which offers the advantages both of readily crossing cell membranes and of doubling the amount of NH_3 carried. In vertebrates, glutamine synthetase forms glu-

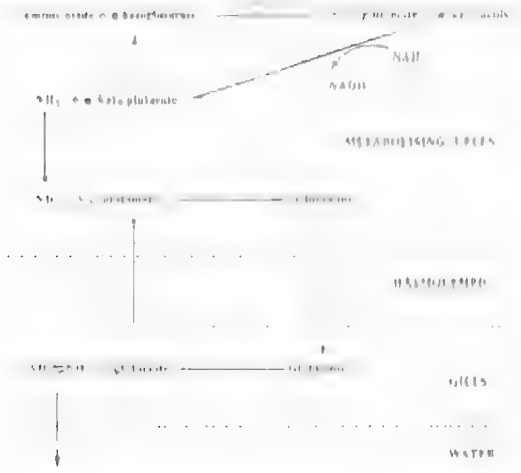

FIG. 2. A pathway for ammonia detoxification and transport in aquatic crustaceans (after King *et al.*, 1985).

tamine in the peripheral tissues and in this form waste is transported to the site of elimination (liver). There is evidence for a similar glutamine detoxification pathway in the crabs *Cancer* and *Carcinus* (Fig. 2) (King *et al.*, 1985). High levels of glutaminase in the gills of these crabs are presumed to release NH_4^+ from glutamine for elimination whilst at the site of ammonia generation (muscle), the levels of glutamine synthetase and glutamate dehydrogenase are high. Glutamine also appears to be an important vehicle for ammonia transport in *Paratelphusa hydrodromous* in freshwater (Krishnamoorthy and Srihari, 1973). This pattern does not hold for all crustaceans, however, as King *et al.* (1985) found little evidence to indicate glutamine detoxification in either *Homarus* or *Pandalus*. Evidence favours GDH as the controlling step in ammonia excretion (summarised in King *et al.*, 1985).

The formation of alanine as a detoxification and transport molecule is also possible as crustaceans possess the necessary enzyme (alanine transaminase) and indeed show high levels of alanine synthesis *in vivo* (Claybrook, 1983). There is at present no specific evidence indicating its general use for detoxification/transport purposes in crustaceans although it is important in vertebrates (Lehninger, 1982). Serine has been implicated as the main route of detoxification in the crayfish *Cherax destructor* (Fellows and Hird, 1979) and a serine cycle has been suggested in other animals (Bishop, 1976).

Other major routes of detoxification commonly seen in animals are *de novo* synthesis of purines and urea production. As the crustaceans do not appear to possess a functional urea cycle (Claybrook, 1983), synthesis of this compound from ammonia is unlikely. Although many crustaceans show periodically high levels of urea in the haemolymph (up to 28 mmol/L in *Holthuisana transversa*, P. Greenaway, unpublished) and excrete some urea, it is generally a minor component of total output. The ability to synthesise purines *de novo* is also thought to be lacking in aquatic crustaceans. The midgut gland is reported to form and excrete spherules of uric acid in several species of aquatic decapods (Fischer, 1926). The small amounts of uric acid excreted or stored are generally thought to be derived from purine catabolism rather than synthesis but this may need to be examined more closely.

ELIMINATION OF WASTE NITROGEN

AQUATIC CRUSTACEANS

There is conclusive evidence from whole animal studies that ammonia is the chief excretory product of aquatic crustaceans and that the site of excretion is the gills. Gills provide a large and well ventilated surface and the respired water carries away excreted ammonia, preventing development of gradients adverse to excretion. Waste nitrogen may arrive at the gills as ammonia (usually 99% NH_4^+ and 1% NH_3) or in detoxified forms such as glutamine and perhaps other amino acids (see above).

The processes by which ammonia is excreted have attracted considerable attention, although much of it has been concerned with osmoregulatory mechanisms rather than excretion (Evans and Cameron, 1986; Schoffeniels, 1976). Whilst the possible mechanisms of elimination of ammonia are now fairly clear, the actual mechanisms used by particular species are seldom so. It is often extremely difficult technically to distinguish between potential excretory mechanisms, and it is frequently unclear whether apparent mechanisms are primarily osmoregulatory or excretory in function as the two systems may be closely linked. Available data for crustaceans are often fragmentary and patterns generally have to be constructed from inadequate data by comparison with better understood mechanisms in fish. Potential mechanisms of excretion are presented in Fig. 3 and discussed below.

The high diffusibility of NH_3 allows loss to the water by diffusion down its partial pressure gradient either through or between the epithelial

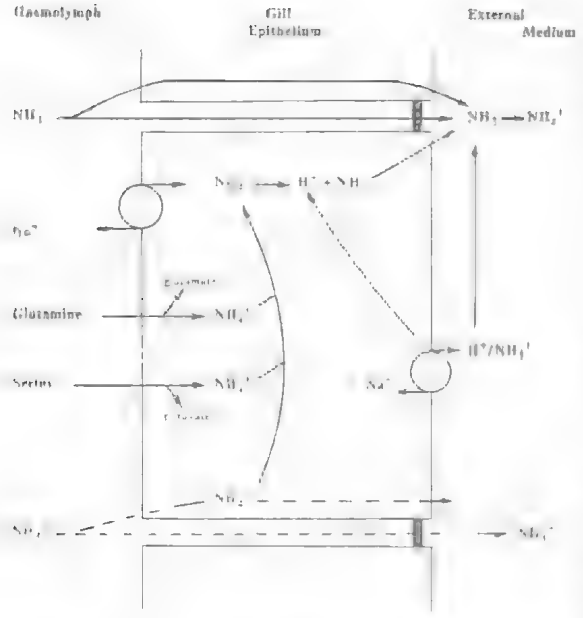

FIG. 3. Diagram summarising possible mechanisms of ammonia elimination in aquatic crustaceans.

cells. The driving gradient will be small as NH_3 forms only a small portion of total ammonia in the haemolymph (Kormanik and Cameron, 1981a). Only a small proportion of waste is likely to be lost in this way unless the gradient can be increased locally by active means (see below). Nevertheless, diffusive loss appears to be the major mechanism in the crab *Callinectes sapidus* in seawater (Kormanik and Cameron, 1981b).

Ammonium ions may also be lost directly from the haemolymph by diffusion down their electrochemical gradient. Passive, transmembrane diffusion may be impeded by the electrical charge of the ion and the pathway taken is probably paracellular, i.e. through intercellular channels and the apical junctions (Kormanik and Cameron, 1981a). There is no information on the importance of this route in crustaceans but it is likely to be minor.

As passive loss of ammonia seems to explain only a small portion of the total excretion of ammonia, the bulk must involve an active component of transport either into or out of the epidermal cell. Entry into the epithelial cells of the gill could be as NH_3, NH_4^+, or detoxified ammonia (amino acids). The neutral amino acids (glutamine particularly, serine and alanine if used) would cross the cell membrane readily but the negatively charged glutamate may require a transport mechanism. Ammonia could then be generated by removal of the amide N of glutamine, deamination of serine and transamination of other amino acids. NH_4^+ could be transported directly into the cell by Na/K activated ATPase with NH_4^+ substituting for K^+ (Towle and Holleland, 1987). NH_3 could enter by diffusion if the gradient were suitable but with active generation of ammonia in the gill epithelium the gradient may well be adverse.

The next step is the elimination of ammonia from the gill epithelial cells. The mechanisms proposed to account for this rely on amiloride sensitive entry into the cell of Na^+ from the water via sodium channels in the apical membrane (Kirschner, 1983). This is thought to create a potential gradient which favours efflux of either NH_4^+ or H^+, again through apical ion-selective channels or antiport mechanisms (Fig. 3) and results in a coupled 1:1 exchange of Na^+ with NH_4^+ or H^+. The ultimate driving force is the operation of basolaterally located Na/K ATPase (Towle and Kays, 1986) which maintains a low intracellular [Na] which allows continued apical influx of Na. Where Na^+/NH_4^+ exchange occurs, the ammonia would be eliminated directly. In the case of Na^+/H^+ exchange the loss of protons would encourage the dissociation of NH_4^+ yielding H^+ and NH_3 and the latter would be lost to the water down its partial pressure gradient (Fig. 3). If osmoregulatory requirements for uptake of Na^+ exceeded the supply of H^+ excretory ammonia, additional protons could be provided from bicarbonate via the activity of carbonic anhydrase.

There is evidence for Na^+/NH_4^+ exchange (but not for Na^+/H^+) in the marine crabs *Cancer* and *Petrolisthes* although it does not account for all the ammonia excreted (Hunter and Kirschner, 1986). In *Uca tangeri*, there is evidence for an apical H^+ pump (Krippeit-Drews *et al.*, 1989) whilst Bigalke (1986) has demonstrated a Na^+/H^+ exchanger in *Eriocheir sinensis*. A Na^+/NH_4^+ exchange has also been suggested in freshwater crayfish (Shaw, 1960). In *Eriocheir sinensis*, a small component of sodium influx apparently exchanges for NH_4^+ (Gilles and Pequeux, 1986) but in the absence of whole body flux data for Na^+ and NH_4^+ it is not clear what proportion of total N excretion this could represent. If nitrogenous excretion is normally much lower than Na influx perhaps all N could exit by this mechanism. In seawater-adapted *E. sinensis*, however, sodium influx was absent, so that ammonia in seawater animals must have been elim-

inated by other means (Gilles and Pequeux, 1986), presumably diffusion. In *Callinectes sapidus*, there is no evidence of Na^+/H^+ coupling (Pressley *et al.*, 1981) and ammonia efflux is also insensitive to amiloride indicating a lack of Na^+/NH_4^+ coupling (Kormanik and Cameron, 1981b). It is suggested that ammonia output in this species is by diffusion of NH_3.

There are then, numerous possible routes by which waste ammonia can be removed, and considerable variability in the way these routes are actually used by particular crustaceans or even within a single species under different conditions. The osmoregulatory requirements for Na^+ transport may well determine which pathways are used and, in euryhaline species, salinity may determine which is used at a particular time. A probable pattern for freshwater crustaceans is described above utilising electrical coupling of Na^+ and H^+, but in marine crustaceans where there is no osmoregulatory requirement for Na^+ a different pattern might be expected.

Other Forms of Excretion

Although ammonia is the dominant excretory product there are some data indicating low levels of excretion of other materials by aquatic Crustacea. Thus uric acid spherules are formed in cells of the midgut gland and excreted into the gut lumen in several species of crabs, the anomuran *Porcellana* and in *Panulirus* (Fischer, 1926). In *Callinectes sapidus* exposed to low temperature and salinity, crystals of uric acid appear in the cells and lumina of the labyrinth and bladder of the excretory organs (Johnson, 1980). Whether this uric acid is derived from an excess of other purines or synthesised by the animals is unknown.

Urea appears to be produced in response to elevated salinity in some crustaceans (Sharma, 1966, 1968, 1969; Krishnamoorthy and Srihari, 1973) and Horne (1968) has suggested that *Cardisoma guanhumi* may excrete urea. Further studies are required on the metabolic capabilities of these animals.

TERRESTRIAL CRUSTACEA

In terrestrial arthropods the major excretory products are purines and clearly there has been strong selective pressure for purinotelism in the terrestrial habitats. It might be expected that the terrestrial crustaceans would conform to this pattern but the limited information available indicates that this is not generally the case.

Isopoda

The isopods are the most successful of the terrestrial crustaceans with approximately 1000 species ranging from supralittoral to desert habitats. Like their aquatic relatives, they are primarily ammonotelic but with the important difference that much of the ammonia excreted is in gaseous form (Dresel and Moyle, 1950; Hartenstein, 1968; Wieser *et al.*, 1969; Wieser and Schweizer, 1970; Wieser, 1972b). Surprisingly, there have been no comprehensive investigations either on the partitioning of excretion between the possible routes or the proportions of various compounds eliminated by each route. Dresel and Moyle (1950) partitioned faecal nonprotein nitrogen in several species. Ammonia was the main nitrogenous product, uric acid made up 5–10%, amino acids 1–6% with only traces of urea. Faecal ammonia in *Porcellio scaber*, however, comprises only 10% of total ammonia excretion, the other 90% being released as a gas (Wieser and Schweizer, 1970). No data are available either for the urinary flow rate nor the concentration of nitrogenous excretory products of urine. Nevertheless, the overall dominance of gaseous NH_3 excretion is clear.

Interestingly, excretion of NH_3 is not continuous but varies diurnally with peak excretion coinciding with periods of minimal activity. It is argued that this allows excretion whilst the animal is resting in a moist microclimate thus minimising water loss during volatilization of NH_3 (Wieser *et al.*, 1969; Wieser, 1972b). This diurnal pattern of excretion may be endogenous in isopods as it is also seen in aquatic species (Kirby and Harbaugh, 1974). Excretion in terrestrial isopods also shows seasonal patterns and is affected by temperature, presumably via the effect on general metabolism (Wieser *et al.*, 1969; Wieser, 1972a, b).

Although it has been known for 40 years that volatilisation of NH_3 is the principal method of nitrogenous excretion in terrestrial isopods, the exact nature of the mechanism involved remains obscure. Given that excretion of NH_3 is not continuous, storage of ammonia between periods of elimination must occur and some method of detoxification of ammonia is indicated. However, data for routine levels of ammonia (*c.* 1.5 $mmol.L^{-1}$) and amino acids (*c.* 2 $mmol.L^{-1}$) in the haemolymph are available only for *P. scaber*, while ammonia concentrations of 1–17 $mmol.L^{-1}$ and glutamate and glutamine of 6–7 $mmol.L^{-1}$ are reported in homogenates of the body wall of *P. scaber* (Wieser and Schweizer, 1972). Infor-

mation on detoxification mechanisms is lacking for terrestrial isopods but the presence of glutamine and glutaminase in the body wall of *P. scaber* indicates a capacity for generation of ammonia from glutamine at this site (Wieser, 1972c; Wieser and Schweizer, 1972). Thus glutamine may be involved in ammonia detoxification and transport as indeed may other mechanisms suggested above for aquatic crustaceans. Further investigations are needed on this matter. Hartenstein (1968, 1970) provided some evidence for oxidative deamination in *Oniscus asellus* but amino-oxidase activity was concentrated in the hepatopancreas which is not suitably located for volatilisation of resultant NH_3.

As ammonia generated will be largely ionised at normal pH levels, the next matter for consideration is its conversion to NH_3. One possibility is that NH_3 is generated in the tissues, at the point of elimination, by local alkalinisation and the NH_3 diffuses out into the air down its partial pressure gradient. Alternatively, NH_4^+ may be eliminated into urine in the excretory organs or across the body wall into fluid (urine or pleopod fluid) which is then made alkaline (Wieser, 1972b) forming NH_3 which is lost to the air. This could be achieved by reabsorption of protons. This would presumably require ion transport across the epidermis lining the channels although it could also occur across the pleopods which have an appropriate ion-transporting epithelium (Kuemmel, 1981).

A potential mechanism for production of gaseous NH_3 which utilises the latter form of elimination was suggested by Hoese (1981). The terrestrial isopods possess a network of water-conducting channels which carry urine. Urine released into this system, from the maxilliary glands, flows along the channels on the dorsal and ventral surfaces and over the pleopods before being re-ingested at the anus (Hoese, 1981, 1982) and it was suggested that volatilisation of NH_3 would be effected during this circulation. If release of urine occurred during periods of inactivity in retreats, water loss from the system by spillage and evaporation would be minimised. This hypothesis is based on observations of micro-anatomy and fluid flow and requires physiological evidence to substantiate it. Thus it must be shown that the urine released contains adequate amounts of NH_4^+ to explain measured rates of NH_3 release. Secondly, it must be demonstrated that the fluid in the channels actually becomes alkaline as gaseous NH_3 cannot be released otherwise.

The terrestrial isopods examined all have deposits of uric acid in the body (Dresel and Moyle, 1950). There are no specific tissues in which this is located, as in the freshwater *Asellus* (Lockwood, 1959), and in *Oniscus asellus* deposits are reported as being located in the 'body wall' (Hartenstein, 1968). The highest level reported is in *Armadillidium* (*c.* 5μmol.g^{-1} wet weight) whilst levels in *O. asellus* and *P. scaber* are almost an order of magnitude lower (Dresel and Moyle, 1950; Hartenstein, 1968). It is not clear whether these deposits represent excess dietary intake of purines or if they have been synthesised *de novo* but there are no data suggesting the presence of synthetic pathways and Hartenstein (1968) considered synthesis unlikely. As uricolytic enzymes are present in *O. asellus* (Hartenstein, 1968) storage of uric acid cannot be considered obligatory and must perform some useful metabolic role.

Amphipoda

There are numerous species of terrestrial amphipods, in the Family Talitridae, ranging from supra-littoral to fully terrestrial. The latter are best represented in the Southern Hemisphere, particularly Australia and New Zealand (Hurley, 1968) where they are extremely abundant in forest and grassland habitats well inland. Knowledge of their nitrogenous excretion is poor. In the supra-littoral *Orchestia*, ammonia makes up the major portion of faecal non-protein nitrogen (Dresel and Moyle, 1950) but it is not known what portion of total nitrogen excretion this represents. Neither is it clear from this work if *Orchestia* excretes gaseous NH_3. Further work is needed on this group, particularly on the terrestrial species.

In the freshwater cave dweller *Niphargus*, spherules of uric acid are stored in special cells on the pericardial septum where the urates bind a variety of anions and cations (Graf and Michaut, 1975). These are considered to be reserves of purine and ions for metabolic usage rather than deposits of waste nitrogen derived from protein catabolism (Graf and Michaut, 1975). Data are lacking for terrestrial species.

Anomura

The Anomura are represented on land by the Coenobitidae, and include the Robber Crab, *Birgus latro*, and twelve species of shell-carrying hermit crabs, *Coenobita*, some of which occupy supra-littoral niches whilst others range inland (Hartnoll, 1988). Nitrogenous excretion has

TABLE 2. The chief excretory products and their routes of excretion in *Gecarcoidea natalis*, *Geograpsus grayi* and *Birgus latro*.

Route	% Total N output	% N Output as Ammonia	% N Output as Uric acid
Gecarcoidea natalis			
Faeces	19.0	67.03	
P	69.9	73.7	
Gas	11.1	100.0	
Total	-	91.8	2.95
Geograpsus grayi			
Faeces	12.3	27.6	
P	5.5	63.3	
Gas	63.3	100.0	
Total		90.9	0.75
Birgus latro			
Faeces	96.2	11.9	82.6
P	0.0012		
Gas	3.8	100.0	
Total		15.2	79.5

been studied only in *B. latro* (Greenaway and Morris, 1989).

B. latro is uricotelic and solid uric acid, excreted in the faeces, comprises about 80% of total non-protein nitrogenous excretory waste. The remaining faecal excretory nitrogen is largely ammonium (11.9%) with some free amino acids and urea (Greenaway and Morris, 1989). Loss of nitrogen in the excretory fluid or as gaseous NH_3 is insignificant (Table 1).

Uric acid appears as separate white portions of the faecal string, quite separate from the brown faeces comprised of food residues. Excretion is episodic with uric acid released into the gut in distinct bouts of excretion. Homogenates of the midgut gland contain xanthine oxidase, the enzyme responsible for the final step in the conversion of purine bases to uric acid and the midgut is presumed to be the active site in the final stage of uric acid production. *B. latro* can synthesise purines *de novo* but nothing is known of the metabolic pathways concerned.

Starved *B. latro* excrete uric acid at an undiminished rate and may metabolise protein during inanition. The observed output could also result from excretion of uric acid stored within the body (see below).

Large amounts of uric acid are stored by *B. latro* particularly in laboratory-maintained animals (Greenaway and Morris, 1989). Extensive white deposits occur in all tissues but it is not clear whether the urate is free in the haemocoel, or is contained in special 'urate' cells as described for the amphipod *Niphargus* (Graf and Michaut, 1975). White deposits have also been observed in *Coenobita brevimanus* (P. Greenaway, unpubl. obs.). The functional significance of urate reserves is unknown but, given that *B. latro* can excrete uric acid, their maintenance must perform some useful metabolic role. This may be to act as a mobilisable nitrogen reserve for synthesis of amino acids or perhaps as an ion store prior to ecdysis or modulation of haemolymph ions during dehydration.

Brachyura

The land crabs are drawn from some 16 different families and range from amphibious to highly terrestrial in habit (Bowman and Abele, 1982; Hartnoll, 1988).

Gecarcinidae. Early work on *Cardisoma guanhumi* discounted the urine as a significant vehicle for excretion (Horne, 1968; Gifford, 1968). Subsequent work has confirmed low urinary nitrogen concentrations (Wolcott and Wolcott, 1987a; Wolcott D.L., pers. comm.; Greenaway and Nakamura, in press) but this has little relevance to excretion as urine in land crabs is not the final excretory fluid and is passed into the branchial chambers. Here it may be extensively modified before being released as a final excretory fluid (P) (Wolcott and Wolcott, 1984, 1985; Greenaway *et al.*, 1990) in which $[NH_4^+]$ is considerably elevated (Wolcott and Wolcott,

1987b; Greenaway and Nakamura, in press). This NH_4^+ must be added to the urine during its residence in the branchial chambers, presumably via the gills but the mechanism is unknown. Any of the mechanisms described above for aquatic crustaceans could be utilised with the urine providing ions for exchange (Fig. 4). The maximum $[NH_4^+]$ recorded in P of *G. natalis* was 73 mmol.L^{-1}, but the average was much lower at c. 11 mmol.L^{-1} (Greenaway and Nakamura, in press) and similar values are reported for other gecarcinids (Wolcott, D.L., pers. comm.).

In *Gecarcoidea natalis*, the chief excretory product is ammonia which makes up 92% of the non-protein nitrogen excreted. This is eliminated largely in the excretory fluid (70%) and the remainder exits as gaseous NH_3 and faecal nitrogen (Greenaway and Nakamura, in press; Table 2). The small amount of excretion of gaseous NH_3 (Table 2) may well have originated from faeces and/or P by direct volatilisation. No significant excretion of gaseous NH_3 was reported from *C. carnifex* (Wood and Boutilier, 1985) and while Horne (1968) reported considerable gaseous excretion by *C. guanhumi* no supporting data were offered.

Routine excretion in gecarcinids is as NH_4^+ in the final excretory fluid. Total nitrogen excretion is relatively low (Horne, 1968; Gifford, 1968; Wolcott and Wolcott, 1987b; Greenaway and Nakamura, in press) and the $[NH_4^+]$ of P is normally low enough to avoid toxicity problems although blood levels are quite high (Table 1).

All species of gecarcinids studied contain deposits of uric acid (Gifford, 1968; Wood and Randall, 1981; Greenaway and Nakamura, in press). Amounts are very variable but appear to be related to diet and accumulation is greatest in laboratory specimens (Wolcott and Wolcott, 1987b). Gifford (1968) considered that uric acid deposits exist free in the haemocoel but histological evidence for this is lacking. The origin of the deposits is unclear but it is unlikely that they are derived solely from excess dietary purine compounds and *de novo* synthesis must be a possibility. As with *Birgus latro* some useful metabolic function of the reserves must be assumed as most crustaceans possess uricolytic enzymes and could degrade the deposits and excrete ammonia.

GRAPSIDAE. The only other land crab in which nitrogenous excretion has been studied is *Geograpsus grayi*, a small carnivorous species with a relatively high rate of nitrogenous excretion (c. 100 umol.kg^{-1}. h^{-1}) (Greenaway and Nakamura, in press). *G. grayi* is also ammonotelic but differs from the gecarcinids in excreting most of its waste nitrogen as gaseous NH_3 (Table 2). Release of gas is not continuous and the crab alternates between bouts of excretion lasting several days and similar periods of minimal excretion. It is probable that protein catabolism is ongoing so ammonia must be detoxified and temporarily stored between bouts of excretory activity. Possible metabolic pathways were outlined above. Excretion of gaseous NH_3 by starved animals is similar to that of fed crabs indicating continued catabolism of protein during starvation (Greenaway and Nakamura, in press). Elimination of NH_3 probably occurs in the branchial chambers where the gills provide a large surface area of epithelium with an ultrastructure suited for ion transport (Greenaway and Farrelly, 1990) and the urine again could provide a source of ions to fuel any exchange processes which may be involved. Ammonia could be lost directly in gaseous form or passed into the P as NH_4^+ and lost as NH_3 following alkalinisation.

The possibility of conflicts between ion regulatory requirements and nitrogen excretion should be considered in brachyurans. The mechanisms of elimination of ammonia described above (Fig. 4), depend on activity of basolateral Na/K–ATPase and the entry of sodium ions from the fluid bathing the gills (in this case urine). In salt replete animals, sodium is not reabsorbed from the urine (Wolcott and Wolcott, 1985; Taylor, Greenaway and Morris, unpubl. data) and this could block ammonia excretion. Greatest difficulty would be experienced by animals on a diet rich in both salt and nitrogen. Dehydration, too, could cause excretory problems as reduction and cessation of flow of excretory fluid would first elevate $[NH_4^+]$, perhaps to toxic levels, and finally eliminate excretion. Such reduction in urine flow occurs during desiccation in the gecarcinids (Kormanik and Harris, 1981). In *Cardisoma carnifex*, ammonia excretion ceases during desiccation but is elevated on rehydration suggesting interim storage in non-toxic form (Wood *et al.*, 1986). Uric acid, amino acids or protein could be utilised for this purpose. These problems could also occur in isopods if excretion relies on ammonium being excreted into the urine or across the gills into a urine or water film.

ASTACIDEA

There are numerous species of semi-terrestrial freshwater crayfish in Australia and N. America

which may spend long periods without free water, e.g. *Engaeus, Cherax*. Nothing is known of their excretory patterns during aerial exposure.

CONCLUSIONS

In aquatic crustaceans, the main excretory product is ammonia which is excreted across the gills into the respiratory water stream and carried away. This ensures that the external ammonia concentration is always low and there is a favourable gradient for excretion and no problems of toxicity. By contrast terrestrial arthropods, other than crustaceans, are mostly purinotelic and the overriding selective factor favouring this, is believed to have been the conservation of body water. Purines may be excreted in crystalline form in the faeces with minimum accompanying water loss, an important factor in the maintenance of water balance of small animals in a desiccating environment. Of the terrestrial crustaceans examined only one species is clearly purinotelic (*Birgus latro*) and, although many more species seem to have an active purine metabolism, their dominant excretory product is ammonia. Comparatively few terrestrial species have been examined and while further work may reveal a few additional purinotelic species, the group appears to be predominantly ammonotelic.

It is of interest to consider how far the various groups of terrestrial crustaceans have diverged from the typical aquatic pattern and moved towards the terrestrial system. In this we are hampered by the relative lack of information on excretion in terrestrial groups.

The gecarcinid crabs appear to have diverged least from the aquatic pattern. They continue to excrete ammonium across their gills and as respiratory water is not available they utilise the excretory fluid from the antennal organs. The volume of urine produced is limited, however, and this necessitates quite high concentrations of ammonia in the branchial chambers and may result in an adverse electrochemical gradient across the gill epithelium. Urine flow is tied to overall water balance rather than excretory requirements and this may cause excretory difficulties necessitating temporary storage of waste nitrogen in negative water balance.

Geograpsus grayi and terrestrial isopods have departed further from the aquatic pattern and have solved the loss of the respiratory water as an excretory vehicle in a different manner. They retain the respiratory medium as a vehicle for excretion by releasing gaseous NH_3. Air flow over the body or through the branchial chambers effectively prevents the buildup of ammonia next to the excretory surface maintaining a favourable gradient for excretion. Until this mechanism of excretion is better understood it will not be possible to say how much independence from water it allows. Actual elimination of NH_3 may first require transport of NH_4^+ into the excretory fluid for conversion to NH_3 or may be direct from the excretory epithelium. Excretion of gaseous NH_3 may be seen as an advance in terrestrial adaptation over that shown by gecarcinids.

Excretion in *Birgus latro* shows a complete break from the aquatic pattern of ammonotelism. The elimination of nitrogenous waste as uric acid and the associated ability to synthesise purine is a major evolutionary advance and places this species at a similar level of adaptation to the other major groups of terrestrial arthropods.

Why have terrestrial crustaceans retained ammonotelism whilst other terrestrial arthropods have not? The main considerations may be the restricted metabolic pathways present at the onset of terrestrial life and the period of evolution spent on land. As aquatic species are ammonotelic and probably lack synthetic pathways for both purines and urea, colonists would thus begin terrestrial life excreting ammonium across the gills and this would be expected to influence both behaviour and habitat selection. Moist microclimates such as leaf litter and rainforest would be favoured and activity restricted to moist conditions so that adequate excretory fluid would be available for elimination of ammonia. Such habitats are characteristic of terrestrial amphipods, isopods and crabs, and although several species live in xeric habitats, their activity is restricted and the animals have a humid retreat or burrow. It would be naive, however, to assume that limitations of the excretory system are responsible for the restricted habitats occupied e.g. *Birgus latro* has a system well tailored for terrestrial life but occupies similar habitats to other terrestrial crabs. Limitations of the reproductive and osmoregulatory systems under terrestrial conditions could equally well affect distribution. Given the habitat in which the animals are found, excretion of various forms of ammonia is feasible and may be advantageous as it carries a minimal energetic cost and requires only small changes to existing systems whilst efficiently excreting waste nitrogen

The apparent lack of synthetic pathways for excretory materials may mean that a long period of evolution on land and strong selective pressure to penetrate drier habitats is necessary before typical terrestrial patterns of excretion can be developed. Fossil evidence, although fragmentary, suggests that most groups of terrestrial crustaceans are relatively recent (and in some cases ongoing) colonists of land (Warner, 1977; Little, 1983).

The mechanisms of detoxification, transport and elimination of nitrogenous waste all require further study, particularly in the terrestrial species. In particular the significance of uric acid deposits is far from clear and we need to clarify both this aspect and the origins of the uric acid (dietary or synthesised?). The occurrence of purinotelism should also be investigated and investigations of the xeric-adapted forms such as the desert isopods *Hemilepistus* and *Venezillio*, the hermit crabs *Coenobita clypeatus* and *C. rugosus*, and the brachyurans *Holthuisana transversa* and *Potamon potamios* may be rewarding. Additionally, some terrestrial groups have not been studied at all (Amphipoda and crayfishes) and should reward future investigation.

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LITERATURE CITED

BATREL, Y. AND REGNAULT, M. 1985. Metabolic pathways of ammoniogenesis in the shrimp, *Crangon crangon* L.: Possible role of glutamate dehydrogenase. Comparative Biochemistry and Physiology **82B**: 217–222.

BIDIGARE, R.R. AND KING, F.D. 1981. The measurement of glutamate dehydrogenase activity in *Praunus flexuosus* and its role in the regulation of ammonium excretion. Comparative Biochemistry and Physiology **70B**: 409–413.

BIGALKE, T. 1986. 'Charakterisierung der (Na^+, K^+)-ATPase und des Na^+/H^+ — Austauschers aus dem ionentransportierenden kiemenepithel der Wollhandkrabbe *Eriocheir sinensis*'. Dissertation, FU Berlin.

BINNS, R. 1969. The physiology of the antennal gland of *Carcinus maenas* (L.). V. Some nitrogenous constituents in the blood and urine. Journal of Experimental Biology **51**: 29–40.

BISHOP, S.H. 1976. Nitrogen metabolism and excretion: regulation of intracellular amino acid concentrations. 414–431. In M. Wiley (ed.) 'Estuarine processes'. (Academic Press: New York).

BOWMAN, T.E. AND ABELE, L.G. 1982. Classification of the recent Crustacea. 1–27. In L.G. Abele (ed.) 'The biology of Crustacea'. Vol. 1. (Academic Press: New York).

CAMPBELL, J.W. 1973. Nitrogen excretion. 279–316. In C.L. Prosser (ed.) 'Comparative animal physiology'. 3rd Edition. (W.B. Saunders Co.: Washington).

CHAPLIN, A.E., HUGGINS, A.K. AND MUNDAY, K.A. 1965. Ionic effects on glutamate dehydrogenase activity from beef liver, lobster muscle and crab muscle. Comparative Biochemistry and Physiology **16**: 49–62.

CLAYBROOK, D.L. 1983. Nitrogen metabolism. 163–213. In L.H. Mantel (ed.) 'The biology of Crustacea', Vol. 5. (Academic Press: New York).

DRESEL, E.B. AND MOYLE, V. 1950. Nitrogenous excretion in amphipods and isopods. Journal of Experimental Biology **27**: 210–225.

EVANS, D.H. AND CAMERON, J.N. 1986. Gill ammonia transport. Journal of Experimental Zoology **239**: 17–23.

FELLOWS, F.C.I. AND HIRD, F.J.R. 1979. Nitrogen metabolism and excretion in the freshwater crayfish *Cherax destructor*. Comparative Biochemistry and Physiology **64B**: 235–238.

FISCHER, E. 1926. L'hepatopancreas des Crustaces excrete des substances puriques. Comptes Rendus Hebdomaires des Sèances de la Societé de Biologie **95**: 1476–1478.

GIFFORD, C.A. 1968. Accumulation of uric acid in the crab *Cardisoma guanhumi*. American Zoologist **8**: 521–528.

GILLES, R. AND PEQUEUX, A. 1986. Physiological and ultrastructural studies of NaCl transport in crustacean gills. Bolletino Zoologica **53**: 173–182.

GRAF, F. AND MICHAUT, P. 1975. Chronologie du developpement et evolution du stockage de calcium et des cellules a urates chez *Niphargus schellenbergi* (Karaman). International Journal of Speleology **7**: 247–272.

GREENAWAY, P. 1971. Calcium regulation in the freshwater mollusc, *Limnaea stagnalis* (L.) (Gastropoda: Pulmonata) I. The effect of internal and external calcium concentration. Journal of Experimental Biology **54**: 199–214.

1985 Calcium balance and moulting in the Crustacea. Biological Reviews **60**: 425–454.

GREENAWAY, P. AND FARRELLY, C.A. 1990. Vasculature of the gas-exchange organs in air-

breathing brachyurans. Physiological Zoology 63: 117–139

GREENAWAY, P. AND MORRIS, S. 1989. Adaptations to a terrestrial existence by the robber crab, *Birgus latro*, III. Nitrogenous excretion. Journal of Experimental Biology 143: 333–346.

GREENAWAY, P. AND NAKAMURA, T. IN PRESS. Nitrogenous excretion in two terrestrial crabs, *Gecarcoidea natalis* and *Geograpsus grayi*. Physiological Zoology.

GREENAWAY, P., TAYLOR, H.H. AND MORRIS, S. 1990. Adaptations to a terrestrial existence by the robber crab, *Birgus latro*. VI. The role of the excretory system in fluid balance. Journal of Experimental Biology 152: 505–519.

HARTENSTEIN, R. 1968. Nitrogen metabolism in the terrestrial isopod *Oniscus asellus*. American Zoologist 8: 507–519.

1970. Nitrogen Metabolism in Non-Insect Arthropods. 299–385. In J. W. Campbell (ed.) 'Comparative biochemistry of nitrogen metabolism. Vol. 1, The invertebrates'. (Academic Press: London and New York).

HARTNOLL, R.G. 1988. Evolution, systematics and geographical distribution. 6–53. In W.W. Burggren and B.R. McMahon (eds) 'Biology of the land crabs'. (Cambridge University Press: New York).

HOESE, B. 1981. Morphologie und Funktion des Wasser-Leitungssystems der terrestrischen Isopoden (Crustacea, Isopoda, Oniscoidea). Zoomorphology 98: 135–167.

1982. Der Ligia-Typ des Wasserleitungssystems bei terrestrischer Isopoden und seine Entwicklung in der Familie Ligiidae (Crustacea, Isopoda, Oniscoidea). Zoologische Jahrucher Abteilung Fur Anatomie und Ontogenie der Tiere 108: 225–261.

HORNE, F.R. 1968. Nitrogen excretion in Crustacea. I. The herbivorous land crab *Cardisoma guanhumi* (Latreille). Comparative Biochemistry and Physiology 26: 687–695.

HUNTER, K.C. AND KIRSCHNER, L.B. 1986. Sodium absorption coupled to ammonia excretion in osmoconformning marine invertebrates. American Journal of Physiology 251: R957–R962.

HURLEY, D.E. 1968. Transition from water to land in amphipod crustaceans. American Zoologist 8: 327–353.

JOHNSON, P.T. 1980. 'Histology of the blue crab, *Callinectes sapidus*. A model for the Decapoda'. (Praeger: New York). 440p.

KING, F.D., CUCCI, T.L. AND BIDIGARE, R.R. 1985. A pathway of nitrogen metabolism in marine decapod crabs. Comparative Biochemistry and Physiology 80B: 401–403.

KIRBY, P.K. AND HARBAUGH, R.D. 1974. Diurnal patterns of ammonia release in marine and terrestrial isopods. Comparative Biochemistry and Physiology 47A: 1313–1321.

KIRSCHNER, L.B. 1983. Sodium chloride absorption across the body surface: frog skins and other epithelia. American Journal of Physiology 244: R429–R443.

KORMANIK, G.A. AND CAMERON, J.N. 1981a. Ammonia excretion in animals that breathe water: a review. Marine Biology Letters 2: 11–23.

1981b. Ammonia excretion in the seawater blue crab (*Callinectes sapidus*) occurs by diffusion, and not Na^+/NH_4+ exchange. Journal of Comparative Physiology 141B: 457–462.

KORMANIK, G.A. AND HARRIS, R.R. 1981. Salt and water balance and antennal gland function in three Pacific species of terrestrial crab (*Gecarcoidea lalandii*, *Cardisoma carnifex*, *Birgus latro* I. Urine production and salt exchange in hydrated crabs. Journal of Experimental Zoology 218: 97–105.

KRIPPEIT-DREWS, P., DREWS, G. AND GRASZYNSKI, K. 1989. Effects of ion substitution on the transepithelial potential difference of the gills of the fiddler crab *Uca tangeri*: evidence for a H^+-pump in the apical membrane. Journal of Comparative Physiology 159B: 43–49.

KRISHNAMOORTHY, R.V. AND SRIHARI, K. 1973. Changes in the excretory patterns of the freshwater field crab *Paratelphusa hydrodromous* upon adaptation to higher salinities. Marine Biology, Berlin 21: 341–348.

KUEMMEL, G. 1981. Fine structural indications of an osmoregulatory function of the gills in terrestrial isopods (Crustacea: Oniscoidea Cell and Tissue Research 214: 663–666.

LEHNINGER, A.L. 1982. 'Principles of Biochemistry'. (Worth Publishers Inc.: New York). 1011p.

LITTLE, C. 1984. 'The colonisation of land. Origins and adaptations of terrestrial animals'. (Cambridge University Press: Cambridge).

LOCKWOOD, A.P.M. 1959. The extra haemolymph sodium of *Asellus aquaticus* (L. Journal of Experimental Biology 36: 562–565.

PRESSLEY, T.A., GRAVES, J.S. AND KRALL, J.R. 1981. Amiloride-sensitive ammonium and sodium fluxes in the blue crab. American Journal of Physiology 241: R370–R378.

REGNAULT, M. 1987. Nitrogen excretion in marine and freshwater Crustacea. Biological Reviews 62: 1–24.

REGNAULT, M. AND BATREL, Y. 1987. Glutamate dehydrogenase of the shrimp *Crangon crangon* L. Effects of shrimp weight and season

upon its activity in the oxidative and reductive function. Comparative Biochemistry and Physiology **86B**: 525–530.

SANDERS, N.K. AND CHILDRESS, J.J. 1988. Ion repalcement as a buoyancy mechanism in a pelagic deep-sea crustacean. Journal of Experimental Biology **138**: 333–343.

SCHOFFENIELS, E. 1976. Adaptations with respect to salinity. Biochimica Biophysica Acta **41**: 179–204.

SHARMA, M. 1966. Studies on the sources and mechanisms of increased urea production by *Orconectes rusticus* under osmotic stress. Comparative Biochemistry and Physiology **24**: 55–60.

1968. Studies on the changes in the pattern of nitrogenous excretion of *Orconectes rusticus* under osmotic stress. Comparative Biochemistry and Physiology **19**: 681–690.

1969. Trigger mechanism of increased urea production by the crayfish, *Orconectes rusticus*, under osmotic stress. Comparative Biochemistry and Physiology **30**: 302–321.

SHAW, J. 1960. The absorption of sodium ions by the crayfish *Astacus pallipes* (Lereboullet III). The effect of other cations in the external solution. Journal of Experimental Biology **37**: 548–556.

SPEEG, K.V. AND CAMPBELL, J.W. 1968. Formation and volatilization of ammonia gas by terrestrial snails. American Journal of Physiology **214**: 1392–1402.

TOWLE, D.W. AND HOLLELAND, T. 1987. Ammonium ion substitutes for K^+ in ATP-dependent Na^+ transport by basolateral membrane vesicles. American Journal of Physiology **252**: R479–R489.

TOWLE, D.W. AND KAYS, W.T. 1986. Basolateral localization of Na^+ * K^+ - ATPase in gill epithelium of two osmoregulating crabs, *Callinectes sapidus* and *Carcinus maenas*. Journal of Experimental Zoology **239**: 311–318.

WARNER, G.F. 1977. 'The biology of crabs'. (Elek Science: London).

WIESER, W. 1972a. O/N ratios of terrestrial isopods at two temperatures. Comparative Biochemistry and Physiology **43A**: 859–868.

1972b. Oxygen consumption and ammonia excretion *Ligia beaudiana* M.-E. Comparative Biochemistry and Physiology **43A**: 869–876.

1972c. A glutaminase in the body wall of terrestrial isopods. Nature **239**: 288–290.

WIESER, W. AND SCHWEIZER, G. 1970. A reexamination of the excretion of nitrogen by terrestrial isopods. Journal of Experimental Biology **52**: 267–274.

1972. Der gehalt an ammoniak und freien aminosuren sowie die eigenschaften einer glutaminase bei *Porcellio scaber* (Isopoda). Journal of Comparative Physiology **81**: 73–88.

WIESER, W., SCHWEIZER, G. AND HARTENSTEIN, R. 1969. Patterns in the release of gaseous ammonia by terrestrial isopods. Oecologia **3**: 390–400.

WOLCOTT, D.L. AND WOLCOTT, T.G. 1987a. Reprocessing urine at the gill augments nitrogen excretion in the land crab, *Gecarcinus lateralis* (Latreille). American Zoologist **27**: 6A.

1987b. Nitrogen limitation in the herbivorous land crab *Cardisoma guanhumi*. Physiological Zoology **60**: 262–268.

WOLCOTT, T.G. AND WOLCOTT, D.L. 1984. Extra-renal urine modification for salt conservation in the land crab *Cardisoma guanhumi*. American Zoologist **24**: 78A.

1985. Extrarenal modification of urine for ion conservation in ghost crabs, *Ocypode quadrata* Fabricius. Journal of Experimental Marine Biology and Ecology **91**: 93–107.

WOOD, C.M. AND BOUTILIER, R.G. 1985. Osmoregulation, ionic exchange, blood chemistry and nitrogenous waste in the land crab *Cardisoma carnifex*: a field and laboratory study. Biological Bulletin of the Marine Biological Laboratory, Woods Hole **169**: 267–290.

WOOD, C.M., BOUTILIER, R.G. AND RANDALL, D.J. 1986. The physiology of dehydration stress in the land crab, *Cardisoma carnifex*: respiration, ionoregulation, acid-base balance and nitrogenous waste excretion. Journal of Experimental Biology **126**: 271–296.

WOOD, C.M. AND RANDALL, D.J. 1981. Oxygen and carbon dioxide exchange during exercise in the land crab *Cardisoma carnifex*. Journal of Experimental Zoology **218**: 7–22.

ZANDEE, D.I. 1966. Metabolism in the crayfish *Astacus astacus* (L.). Biosynthesis of amino-acids. Archives Internationale de Physiologie et Biochimie **74**: 35–44.